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Short Communication

Identification of promising sources of barley (*Hordeum vulgare* L) for malt beta-glucanase activity and wort beta-glucan content

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Malt is one of the major industrial products from barley, which is further utilized mainly for beer production. The malt producing industry requires certain minimum quality parameters in the barley to get higher recovery and better quality (Kumar *et al.,* 2013). India is one of the emerging markets for barley malt consumption for brewing and nutraceuticals and thus needs malting varieties meeting the international standards. However, the shorter grain filling period in sub-tropical plains of India as compared to the temperate European climates is a major challenge to get the best quality under Indian conditions. Since most of the parameters are governed by genotype, growing environment and cultural practices, but its most important to breed malting barley genotypes with superior quality under Indian climatic conditions (Kumar *et al*., 2017). Several genotypes have been bred in India with better quality, higher grain yield and disease resistance (Kumar *et al*., 2014). Improvement being a continuous process, currently one of the major objectives of the Indian malt barley improvement programme is to breed genotypes with lower wort beta glucans. The higher beta glucans content in grain reduces water uptake during steeping; while in wort it adversely affects filtration rate and quality of malt extract. The ideal malt barley variety should have lower grain beta glucans coupled with higher malt beta glucanase activity, to get lower values of wort beta glucans. Malt beta glucanase is the major enzyme which breaks down beta glucans in endosperm cell walls and its higher activity is always desirable. This study was carried out to identify the potential sources of higher malt beta glucanase and lower wort beta glucans in barley genotypes.

A total of nine genotypes were grown in rabi season (mid-November to mid-April) at Karnal, Hisar, Ludhiana, Pantnagar, Durgapura and Kanpur during 2020-21 with plot size of 2.5 x 0.46 m^2 (two rows of 2.5 m each). The crop was fertilized with 60 kg N (in 2 split); 30 kg P and 30 kg K. The grain samples from each location were received and stored in air tight bags at -20°C till further analysis.

The bold/plump grains (grains retained on 2.5 mm screen of Sortimat, the EBC approved grain uniformity analyser from Pfeuffer Germany) were taken for malting purpose. The malt was prepared in an automatic micro-malting system (Joe White Australia) following the below steeping, germination and kilning schedule.

1. Steeping: 8 hours dip in water (temperature 25°C) with continuous aeration, followed by 16 hours air rest (temperature 18°C) and again 6 hours dip in water (temperature 18°C) with continuous aeration
2. Germination: 24 hrs at 18°C→18 hrs at 17°C

3. Kilning: 2 hrs at 30°C→ 2 hrs at 45°C→2 hrs at 50°C→1 hr at 55°C→1 hr at 60°C→16 hr at 65°C →1 hr at 70°C →1 hr at 75°C →1 hr at 80°C

The malt was taken out from machine after cooling to room temperature and rootlets removed by gentle hand rubbing. The malt samples were then stored in air tight interlocking plastic bags at -20°C till further analysis. The malt was grounded by an EBC approved Buhler's laboratory Mill at fine grinding setting and powdered malt flour was extracted in IEC make (Australia) mashing bath for 45°C for 30 minutes and then at 70°C for 90 minutes, making a total duration of 120 minutes. The resulting slurry was filtered through Whatman 2555 ½ filter papers and wort was collected and stored at -20°C till further analysis. Beta glucanase activity in the malt was estimated using Megazyme Assay Kit (Megazyme Ireland Ltd.) following the method of McCleary and Shameer (1987). Mixed linkage (1→3; 1→4)-β-D-glucans in grain and wort was measured using Megazyme Assay Kit (Megazyme Ireland Ltd.) following the method of McCleary and Nurthen (1986). The data was analysed with CropStat 7.2.

The higher levels of beta glucans in barley grain may lead to poor modification of the grain as incomplete degradation of endosperm cell wall may hinder the diffusion of enzymes required for degradation of kernel reserves (Habschied *et al.,* 2020). Therefore, barley genotypes with lower grain beta glucans are considered ideal to get better malt extract values. Besides the beta glucans content in the grain, the activity of beta glucanase, which usually develops during grain germination, should be high, to further reduce the wort beta glucan content. (Habschied *et al*., 2020). For breeding improved malt barley varieties, sources of these traits need to be identified. In this preliminary investigation, the genotype ICARDA-11 (382 U/kg malt); ICARDA-9 (379 U/kg malt) and DWRB 197 (374 U/kg malt) have been found to have higher beta glucanase activity (Table 1). Wang *et al.* (2004) reported a range of 313 to 490 U/kg of malt beta glucanase activity and suggested that besides selecting for lower beta glucans genotypes, it's also important to have material with higher malt beta glucanase activity. In our study average values for beta glucanase were close to 400U/kg in the genotypes ICARDA-9 and ICARDA-11, and at Durgapura it was well above 450 for these two genotypes. Han *et al.* (1995) reported genes Glb1 and Glb2 on chromosome 1H and 7H, are encoding for (1,3;1,4)-β-glucanases.

Table 1: Activity of Beta-Glucanase (Units/kg) in malt of different barley genotypes

Genotype	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Average
ICARDA-5	198	241	187	229	287	226	**228**
ICARDA-9	384	316	318	492	415	346	**379**
ICARDA-11	380	360	382	487	369	315	382
BK 306	345	315	390	333	337	238	**326**
DWRB 197	386	354	350	373	429	354	**374**
DWRUB 52 (c)	260	279	309	308	239	280	**279**
DWRB 101 (c)	175	186	197	263	256	140	**203**
DWRB-182 (c)	167	264	257	299	240	167	232
DWR 37 ©	195	223	208	269	272	272	**240**
Average	**277**	**282**	**289**	**339**	**316**	**260**	
LSD 5%							46

The higher values of beta-glucan may lead to poor lautering performance and affect the colloidal stability of the beer. For brewers, the total β-glucan content in wort should be less than 200 ppm (Davis 2006), while there are no specific limits for beta glucan in malt, as they consider it in the final product to be used for brewing. In this study, we recorded two genotypes i.e., ICARDA-9 (153 ppm) and ICARDA-11 (117 ppm), with average values well below 200 ppm in the wort (Table 2). The genotype ICARDA-11 was more consistent as it scored less than 200 ppm at all the locations. It becomes more important as the genotype is six row type (Table 2). Genotypes BK 306 and ICARDA 5 recorded average wort beta glucan values very close to 200 ppm, but the former had higher

beta glucanase activity bringing down wort beta glucan from more higher grain beta glucan, while the second has poor beta glucanase activity, though it has lowest grain beta glucan. Similarly, DWRB 197 had wort beta glucans value of 347 ppm (close or lower than the checks), but has clearly depicted the role of higher beta glucanase activity in degradation of grain beta glucans, which was highest in grain (Table 3). This study has further verified the earlier reported trait of this genotype (Kumar *et al.* 2020) and identified clearly contrasting genotypes for beta glucan contents and beta glucanase activity.

Table 2: Wort Beta-Glucan content (ppm) in different barley genotypes

Genotype	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Average
ICARDA-5	382	178	275	344	78	166	**237**
ICARDA-9	327	139	277	93	33	50	**153**
ICARDA-11	198	92	167	101	39	107	**117**
BK 306	423	169	228	79	142	171	**202**
DWRB 197	737	288	392	350	194	120	**347**
DWRUB 52 (c)	552	397	404	392	261	280	**381**
DWRB 101 (c)	471	370	602	447	232	176	**383**
DWRB-182 (c)	368	325	432	441	234	321	**353**
DWR 37 ©	683	856	633	462	269	239	**524**
Average	460	313	379	301	165	181	
LSD 5%							115

Table 3: Grain Beta Glucan content (% dwb) in different barley genotypes

Genotype	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Average
ICARDA-5	3.9	3.6	4.3	4.1	3.3	3.8	**3.8**
ICARDA-9	3.0	3.7	4.2	4.3	3.7	4.4	**3.9**
ICARDA-11	4.4	4.3	4.1	4.2	4.0	4.1	**4.2**
BK 306	4.3	3.8	4.4	4.7	4.5	4.5	**4.4**
DWRB 197	6.4	6.3	6.6	6.4	6.5	5.9	**6.4**
DWRUB 52 (c)	4.2	5.4	4.4	5.2	5.1	5.3	**4.9**
DWRB 101 (c)	4.4	5.4	5.9	5.0	5.1	5.0	**5.1**
DWRB-182 (c)	4.2	4.2	4.4	4.7	4.0	4.0	**4.3**
DWR 37 ©	4.4	4.8	4.8	5.0	5.4	4.8	**4.9**
Average	4.4	4.6	4.8	4.8	4.6	4.6	
LSD 5%							0.4

Table 4: Parentage of test genotypes

Genotype	Parentage	Row type
ICARDA-5	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI/5/ARUPO/K8755// MORA (ICARDA PYT-15-41)	2
ICARDA-9	J09049 F3 10/030552 (ICARDA PYT 15-93)	2
ICARDA-11	SEN/5/LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI (ICARDA PYT-15-50)	6
BK 306	BK9811 / DL472 (F5 -50)	2
DWRB 197	DWRUB52/DWR84	2

Conclusion

The genotypes ICARDA-11, ICARDA-9 and DWRB 197 have been found to have higher malt beta glucanase activity and ICARDA-11 and ICARDA-9 with lowest wort beta glucans content. These genotypes may serve as potential sources of these traits in Indian malt barley improvement programme towards bringing down the wort beta glucan content as well as for further biochemical and molecular studies on basic aspects of malt quality under sub-tropical climates.

Conflict of Interest

Authors declare that they have no conflict of interest

Ethical Compliance Statement

NA

Author's Contribution

Dinesh Kumar (Conceptualization, planning, experimentation and writing); Ramesh Pal Singh Verma (Conceptualization, planning and major editing); Lokendra Kumar (Breeder of DWRB); Vishnu Kumar 197 (Planning and editing): Gyanendra Pratap Singh (Overall Supervision)

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